

COTULA CORONOPIFOLIA L. Common on muddy borders of brackish marshes and margins of tidal streams. I have observed and collected this species from several stations, but failed to record it earlier. Semiahmoo Bay, 1919; Lummi Point, 1933, 1937, 1943; Point Roberts, 1937; Eliza Island, 1939; Chuckanut Bay, 1939; Neptune Beach and Terrell Lake, 1950.

LACTUCA MURALIS Fresen. Common in the shade under evergreen trees in city park, Lynden; 1950.

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AN ANTHRACNOSE DISEASE OF *UMBELLULARIA CALIFORNICA*¹

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A fungus considered here as belonging to the genus *Kabatiella* was found to be associated consistently with diseased leaves of the California laurel tree, *Umbellularia californica* Nuttall, collected in the coast ranges of central California.

Brown, necrotic patches, originating at the tip, lateral margins, or petiolar region of the leaf, are produced by this fungus and may eventually extend over the whole leaf (pl. 3, fig. A). The affected areas often have a rather roughened appearance due to numerous acervuli which break through the epidermis.

Kabatiella seems to be present in such diseased leaves throughout the year, at least in the rather humid and temperate coastal areas where collections were made. The older leaves (2-3 years old) were most commonly infected with the fungus, although in some cases where branches died back for other reasons, *Kabatiella* was isolated from younger leaves.

TAXONOMY OF THE CAUSAL AGENT

Bubak (1907) established the genus *Kabatiella* on June 8, 1907, naming a new imperfect fungus, *K. microsticta* Bubak, found on *Convallaria majalis* L. A second species, *Kabatiella ribis* Vasil. on black currant, was described by Vassilievsky (1923). Atkinson and Edgerton (1907) erected the genus *Protocoronospora* in September, 1907, and described *P. nigricans* Atk. and Edgert., a pathogenic species found on cultivated vetch, as the type. These authors noted the resemblance of the spores in mass to those of the anthracnose fungi in the order Melanconiales, but regarded the fungus as being a basidiomycete because of the peculiar spore-

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bearing structures. They considered these to be basidia and observed that from four to eight sessile spores were borne in a whorl at the end of these basidia.

Further study was made of this fungus by Wolf (1920) who found cytological evidence confirming that the structures regarded as basidia by Atkinson and Edgerton were conidiophores and that the fungus properly belonged in the order Melanconiales.

In reviewing this group of fungi, Karakulin (1923) was convinced that his *Exobasidiopsis viciae* Karak. described in 1922 is identical with *Protocoronospora nigricans* and that *Kabatiella microsticta*, *K. ribis*, *Pachybasidiella polyspora* Bub. and Syd. and *Gloeosporium caulivorum* Kirch. are all congeneric with the first two above mentioned pathogens. *Kabatiella*, having priority is the generic name to be used. Therefore, Karakulin included the following species in *Kabatiella* and made the necessary transfers: *K. microsticta* Bubak, *K. polyspora* (Bub. and Syd.) Karak., *K. ribis* Vassil., *K. caulivora* (Kirch.) Karak. and *K. nigricans* (Atk. and Edgert.) Karak. Wolf and Wolf (1947, p. 394) apparently accept Karakulin's revision for they state that "the so-called *Protocoronospora nigricans* in vetch appears properly to belong in *Kabatiella*." Sampson (1928) in her studies on the red clover anthracnose employs *Kabatiella caulivora* for the fungus previously designated as *Gloeosporium caulivorum*.

The genus *Protocoronospora* appears, therefore, to be invalid and species formerly placed in that genus should be placed in *Kabatiella*. The original descriptions of both genera appear to apply to similar fungi, the chief distinction being whether the conidia are borne sessile or on sterigmata. The fundamental character common to all the fungi referred to *Kabatiella* by Karakulin is the possession of a conidiophore of the pseudobasidial type. The club-shaped conidiophores arise from a small cushion or large stroma-like hymenial layer and emerge either through the stomata or by rupturing the epidermis. Several hyaline, unicellular conidia are borne terminally or rarely laterally on very minute stalks which are scarcely visible until after the conidia fall. Since even in species long recognized as belonging in *Kabatiella*, the sterigmata are sometimes not visible until after the conidia have fallen from the conidiophore, it is conceivable that they were present in *P. nigricans*, but not observed or not interpreted as such. This character does not in any event seem to justify the separation of two genera so similar in other characteristics.

A fungus found to cause a disease of mistletoe, *Phoradendron flavescens* (Pursh) Nuttall var. *macrophylla* Engelm. was considered by Darling (1940) to be sufficiently distinct from *Protocoronospora nigricans* to warrant its description as *P. Phoradendri*, a second species in the genus. One of the chief differences, aside from the respective host plants, was the fact that the conidia of *P. nigricans* germinate by budding or by germ tubes, while those of

P. Phoradendri were observed to germinate only by a germ tube and never by budding. Darling states in her description of the species that small sterigmata are present, which fact, if used as a generic character would in any event place this fungus in *Kabatiella* rather than in the genus *Protocoronospora*. The following transfer is therefore proposed:

Kabatiella Phoradendri (Darling) comb. nov. *Protocoronospora Phoradendri* Darling, Madroño 5: 242. 1940.

The genus *Aureobasidium* Viala and Boyer described in 1891 has been listed as being synonymous (Ainsworth and Bisby, 1945, Clements and Shear, 1931) with *Kabatiella*, but this has not been confirmed by critical studies. Karakulin, in his discussion of fungi belonging in the genus *Kabatiella* fails to mention *Aureobasidium*. Various authors (Ciferri, 1922) have placed this genus near *Exobasidium*, while others have placed it in the Deuteromycetes. As originally described, the genus (Viala and Boyer, 1891) was considered to belong in the Basidiomycetes. Viennot-Bourgin (1949, p. 609) regards certain species of the form genera *Aureobasidium*, *Dematium*, *Exobasidium* and *Pullularia* as being synonymous, these species possessing *Anthostomella* as the ascigerous stage. This latter group of fungi possesses characters quite different from the fungus considered herein whose characters agree more closely with those of the genus *Kabatiella*.

MORPHOLOGY

Kabatiella Phoradendri was isolated from leaves of mistletoe collected on the northwest shore of Clear Lake, California (January 25, 1950) in order to compare it with the *Kabatiella* isolate from California laurel.

The acervuli of the *Umbellularia* isolate usually break through the lower (occasionally the upper) epidermis of the leaves. Their size varies from 155 to 263 microns in diameter. No setae were observed in the acervuli of this isolate although they are reported to occur in *K. Phoradendri* and *K. nigricans* and are reported as being absent in *K. caulivora*.

The conidiophores are clavate to cylindrical, 21–65 microns long and 7 to 11 microns broad. One to eleven conidia are borne terminally (pl. 4, fig. C) on small obscure sterigmata that are visible only after the conidia are shed.

The conidia of the isolate of *Kabatiella* found on *Umbellularia californica* are 20.1–28.9 microns long and 2.8–3.5 microns broad, average 24.2×3.1 microns. They are allantoid in shape and muticate (pl. 4, fig. B). In contrast, the conidia of *Kabatiella Phoradendri* are 15–26 microns long and 4.5–6.5 microns broad and much more curved, being described by Darling (1940) as falcate (pl. 4, fig. A).

The mycelial hyphae of the *Umbellularia* isolate are 3.2–5.6 microns in diameter.

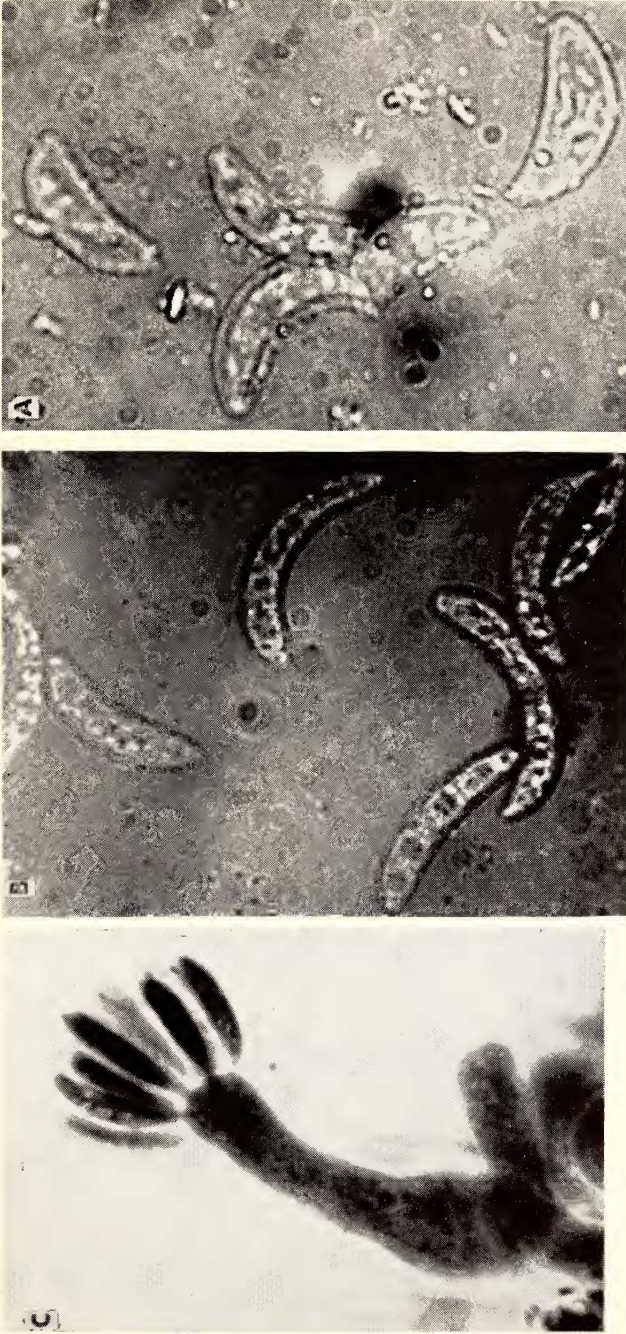


PLATE 4. CONIDIA OF *KABATIELLA*. FIG. A. *Kabatiella Phoradendri* (\times ca. 1160). FIG. B. *Kabatiella Phoradendri* f. *umbellulariae* (\times ca. 1160). FIG. C. Conidiophore and conidia of *K. Phoradendri* f. *umbellulariae* (\times ca. 650).

In old cultures conidia of the *Umbellularia* isolate were observed to give rise to new conidia either at their ends or sides. When this phenomenon occurred the protoplasm from the old conidium moved out into the new one. It is doubtful if one could regard this as a true process of budding in the sense of a yeast budding. In the latter case both resulting cells contain functioning protoplasts. The type of budding which Wolf (1920) describes as occurring in *Kabatiella nigricans* and which Martin (1928, 1929) describes as occurring in *K. microsticta* and *K. polyspora* has not been observed in the isolate from *Umbellularia californica*. When conidia of this fungus were plated out on water agar, they germinated by germ tube only. In this character this isolate seems to resemble *Kabatiella Phoradendri*, but differs from *K. nigricans*, *K. ribis*, *K. microsticta*, *K. polyspora* and *K. caulivora*.

Cultures on potato dextrose agar slants show very slow and sparse growth even when kept at optimum temperatures for growth. The conidia are cream colored in mass and the mycelium, at first hyaline, darkens with age, resulting in colonies which are greyish-white in color on the perimeter, but dark brown to black in the center.

On oatmeal agar slants, growth is greater than on potato dextrose agar under similar environmental conditions. More mycelial growth is made, but the colonies still remain compact and turn dark with age.

On both the above media, greater conidial production was observed if inoculation was made by pouring a spore suspension over the entire surface of the medium rather than by introducing single spores or mass transfers of mycelium.

Growth was good and acervulus formation excellent on a natural medium of sterile pea straw in agar plates prepared according to the method described by Hansen and Snyder (1947). Plates inoculated February 1, 1950, and kept in a rack outside the laboratory window showed well-formed acervuli in six days. On this medium the conidia are pink-colored in mass. Dried leaves of *Umbellularia californica* ground and prepared by the same method proved to be equally as good as the pea straw for the growth of this fungus.

TEMPERATURE RELATIONS

Tests were made to determine the effect of temperature on the growth rate of the isolate from *Umbellularia* and of *Kabatiella Phoradendri*. Single spore cultures on potato dextrose agar were prepared and placed in unlighted temperature chambers at temperatures ranging from 1 to 31° C. The diameters of the colonies were measured at the end of twenty-eight days to determine relative growth rates. The critical temperatures for growth for the isolate from *Umbellularia californica* were 4, 16 and 25° C. (pl. 5, fig. C). Those for the mistletoe isolate were 4, 22,

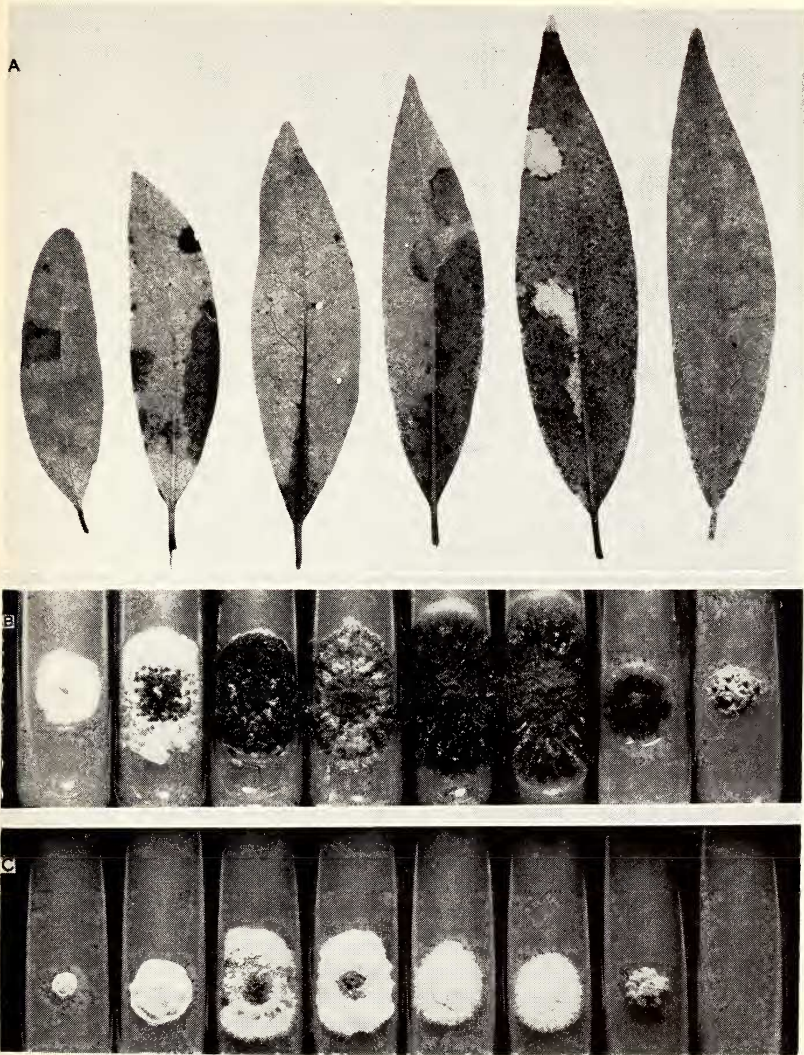


PLATE 5. KABATIELLA. FIG. A. Leaves of *Umbellularia californica* infected with *Kabatiella Phoradendri* f. *umbellulariae*. FIG. B. Cultures of *K. Phoradendri* on potato dextrose agar kept at 7° C. (left) to 28° C. (right) at 3 degree intervals. FIG. C. Cultures of *K. Phoradendri* f. *umbellulariae* (same culture conditions as in B).

and 28° C. (pl. 5, fig. B). Thus the mistletoe isolate can tolerate a somewhat greater range in temperature and grows best at a temperature somewhat higher than the temperature for optimum growth of the isolate from *U. californica*. Considering the regions in which the respective fungi are most commonly found, this difference in behavior toward temperature might be expected. The temperatures are rather moderate in the coastal region from which most of the *Kabatiella* isolations from *Umbellularia californica* were made. On the contrary, *Kabatiella Phoradendri* was isolated from mistletoe plants growing in a region where the fluctuations in temperature are more extreme and the average temperature is somewhat higher.

It was found that the optimum temperature for sporulation was 10° C. for the isolate from *Umbellularia* whereas sporulation was equally great at 10 and 13° C. for *Kabatiella Phoradendri*. In both fungi, sporulation was greatly reduced at higher temperatures.

INOCULATION EXPERIMENTS

Some difficulty was experienced in infecting living leaves of *Umbellularia californica* with *Kabatiella* isolated from this host. Seedlings kept for two days in a moist chamber out of doors were inoculated by spraying with a spore suspension. Some of the leaves had been wounded previously by scratching or pricking with a dissecting needle. These were kept in a moist chamber for two days after inoculation but they failed subsequently to show evidence of infection.

When leaves, dried and sterilized with propylene oxide (Hansen and Snyder, 1947), were placed in a sterile moist chamber and inoculated by spraying with a spore suspension of the fungus, development took place rapidly. At first the mycelium and acervuli appeared to be growing only on the surface of the dead leaves, presumably being nourished by materials which had diffused out from the leaf into the water on the leaf surface. This mycelium had not invaded the tissue and could easily be scraped off with a dissecting needle. Two weeks after inoculation, however, the fungus had invaded the leaf tissue, and numerous acervuli could be observed erupting through the epidermis of the leaf. This would seem to indicate that this isolate of *Kabatiella* is a rather weak parasite, but readily assumes a saprophytic existence.

Detached twigs of *Umbellularia californica*, each bearing several leaves, were placed in moist chambers, the basal end of the twig being immersed in a beaker of water. Some of the leaves were wounded by scratching them with a dissecting needle. Two twigs were inoculated by spraying with a spore suspension of the *Kabatiella* isolate from *Umbellularia californica*, and the third with *Kabatiella Phoradendri*. Two of these moist chambers with contents were placed in unlighted temperature chambers at the opti-

mum temperatures for the respective pathogens, i.e., 13° C. for the *Kabatiella* from *Umbellularia californica* and 22° C. for *Kabatiella Phoradendri*. The remaining chamber, in which the leaves were inoculated with the *Kabatiella* from *Umbellularia californica*, was kept out of doors (March 14, 1950).

At the end of ten days all the inoculated leaves kept in the temperature chambers had become infected. Acervuli were formed abundantly along the wounds in the leaves, and also were formed on unwounded leaves. Acervuli were especially prominent along the advancing margins of infection. The inoculated leaves kept out of doors showed no infection.

LIFE CYCLE

Though the isolate of *Kabatiella* from *Umbellularia californica* was studied on a variety of culture media and on diseased leaves, no perfect stage was ever observed. This was also the experience of Wolf (1920) with *Kabatiella nigricans*. No reference to the discovery of a perfect stage for members of this genus was found by the author. The organism seems to persist in the leaves, however, in a mycelial state producing acervuli readily under proper conditions. Since the leaves remain on *Umbellularia californica* for several seasons, the fungus can maintain itself by sporulating on diseased parts of older leaves, disseminating conidia from these foci of infection to younger leaves from year to year.

Such a hold-over in the vegetative state was demonstrated by Wolf (1920) with *Kabatiella nigricans*. The mycelium of this pathogen can remain alive in old infected parts of vetch and also is seed borne, the mycelium penetrating well into the seed. The longevity of the conidia of *K. caulivora* has been shown by Sampson (1928), the conidia remaining viable on red clover seed for eighteen months after harvest.

DISCUSSION

The characteristics of the fungus isolated from *Umbellularia californica* are most similar to those of fungi placed in the genus *Kabatiella*. As reported in the literature, the species constituting this genus appear to differ chiefly, aside from host relationships, in the manner in which the conidia germinate and in the manner in which they are borne, i.e., sessile or on sterigmata.

The conidia of *Kabatiella nigricans* and *K. polyspora* (Bubak and Sydow, 1915) are reported as being sessile. Those of *K. microsticta*, *K. ribis*, *K. caulivora* and *K. Phoradendri* are reported as being borne on sterigmata, although these are minute and often are apparent only after the conidia are shed. This fact might indicate that sterigmata were present, but not observed or not construed as such in the first two species mentioned. In any event, this does not appear to be a reliable character upon which

to base a differentiation of species.

The conidia of *K. nigricans*, *K. ribis*, *K. microsticta*, *K. polyspora* and *K. caulivora* have been reported to germinate by budding. In *K. Phoradendri* no budding is reported to occur. In this character the isolate of *Kabatiella* from *Umbellularia californica* resembles *Kabatiella Phoradendri* and differs from the other species in the genus.

Though the new isolate resembles *K. Phoradendri* in the above character, the two fungi differ from one another morphologically in the shape and size of the conidia, physiologically in their respective temperature ranges, and in the symptoms they produce on their respective hosts.

The proper placement of a newly discovered fungus in such a genus as *Kabatiella* would involve a detailed study of all the species in the genus and perhaps in closely related genera, a task somewhat beyond the scope of the present study. The isolate from *Umbellularia californica* seems, however, to be related to *Kabatiella Phoradendri* more closely than to the other species in the genus, in so far as can be determined from the literature. Both fungi are found in close proximity, the respective hosts being native to the regions in which collection of diseased specimens was made; in old cultures of the isolate from *Umbellularia californica* the conidia assume a shape which approaches that of the conidia of *Kabatiella Phoradendri*; and in both fungi the conidia are borne on minute sterigmata which are visible only after the spores are shed. Furthermore, *K. Phoradendri* was induced to grow on *Umbellularia* in culture.

Considering the wide range of variability which can occur in a single species, as demonstrated in species belonging to the genus *Fusarium* by Snyder and Hansen (1940), it appears that the differences exhibited by these two isolates might well represent such variation within the species *Kabatiella Phoradendri*. It seems, therefore, on the basis of host relationships, best to regard the isolate from *Umbellularia californica* as a form of *Kabatiella Phoradendri*, rather than as a new species.

KABATIELLA PHORADENDRI Darling f. *umbellulariae* forma nova. A specie conidiis angustioris minus curvatis differt. Habitat in foliis *Umbellulariae* (Lauracearum). America Borealis.

Type. On *Umbellularia californica* Nuttall, north side of Russian River near Monte Rio, Sonoma County, California, November 15, 1949, *John M. Harvey* (Herb. Univ. Calif. no. 924306).

This form has been collected on *Umbellularia californica* in Alameda, Contra Costa, Santa Cruz, Marin, Mendocino, Sonoma, Napa and Lake counties, California.

SUMMARY

A fungus belonging in the genus *Kabatiella* was found to be

consistently associated with diseased leaves of *Umbellularia californica*. Symptoms are brown, necrotic areas which appear near the tip, lateral margins, or base of the leaf.

The pathogen was isolated, studied in culture, and compared with related organisms. Inoculation experiments were carried out to determine the pathogenicity of the fungus.

The position of the closely related fungus, *Protocoronospora Phoradendri* is discussed and its transfer to *Kabatiella* is proposed. The isolate from *Umbellularia californica* is proposed as a form of *Kabatiella Phoradendri*.

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